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PROTON RELAXATION MECHANISMS AND THE MEASUREMENTS OF r_Φ, r_Ψ AND TRANSANNULAR INTERPROTON DISTANCES IN GRAMICIDIN S

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Monoselective, $R_0'(SE)$, biselective, $R_0'(i,j)$, and nonselective proton spin-lattice relaxation rates have been measured for dilute solutions of gramicidin S in dimethyl sulfoxide and used to evaluate cross-relaxation rates ($\sigma' = R_0'(i,j) - R_0'(SE)$) and F' ratios ($F' = R'(NS)/R_0'(SE)$). The cross-relaxation parameters, σ, and F' ratios measured for backbone gramicidin S protons predict that the same correlation time, $\tau_c = 1.2 \times 10^{-9}$ s, modulates all the dipolar proton-proton interactions and that these interactions represent the main source for the proton spin-lattice relaxation process. The larger relaxation rates for amide versus α-protons of the backbone are attributed to dipolar relaxation between ^{14}N and its directly bonded protons and is an approximate measure of the extent of this. The intrabackbone proton-proton distances, evaluated from σ values, were consistent with the antiparallel β-plated sheet/βII′-turn conformation previously proposed for gramicidin S in solution.

1. Introduction

The nuclear Overhauser effect (NOE) [1] has been used to calculate interproton distances and correlation time for gramicidin S [2,3], and quantitatively confirm earlier results [4–7]. Here we exemplify a quantitative study of microenvironments, relaxation mechanisms, internuclear distances and correlation times for protons in biopolymers by using data obtained from spin-lattice relaxation rates measured in the nonselective, selective and biselective modes [8–10]. F' ratios and cross-relaxation parameters, σ, calculated from these proton relaxation rates gave correlation times in agreement with previous determinations [2,3,11]. The r_Φ and r_Ψ vicinal interproton distances are in agreement with the NMR-derived gramicidin S secondary conformation [2,5,11–15].

Other conformations are consistent with the r_Φ and r_Ψ distance reported here and elsewhere [2,3,5] and so it is necessary to have additional criteria as reported here for gramicidin S and elsewhere for tyrocidin A [16–18]. This is the detection of interactions between protons located in remote parts of the polypeptide chain. Here we report a distance of 2.4 Å between the α-protons of ornithine i and ornithine $i+5$ residues, which is a general example of a transannular interproton distance criterion for distinguishing antiparallel and parallel β-pleated sheet conformational moieties and other conformations.

The approach to determining polypeptide conformation from proton relaxation parameters should be applicable to nucleic acid, sugar and larger proteins.

2. Materials and methods

Samples were prepared by dissolving 10 mg gramicidin S, purchased from Sigma, in 100% [$^2H_6$]dimethyl sulfoxide (DMSO-d_6). To avoid the

presence of paramagnetic species in the samples, these were thoroughly degassed to remove dissolved oxygen and a small amount of EDTA was added to the solutions in order the complex paramagnetic metal ions present as impurities. Spectra were obtained with a Bruker WH-270 NMR spectrometer equipped with a Nicolet 1180 computer. To measure mono-, bi- and nonselective relaxation rates, the $(180-\tau-90-T)_n$ pulse sequence was used. The nonselective 180° pulse was typically 20 μs and the selective ones, generated by the decoupler channel, had a duration of 10 ms. In the biselective experiments, the decoupler pulse was frequency modulated by a Hewlett-Packard 3300 A function generator. Partially relaxed spectra were obtained in the selective and biselective modes by using respectively the following arrays of variable τ values (in s) (a) 0.005, 0.010, 0.030, 0.060, 0.100, 0.120, 0.140, 0.160, 0.180, 0.200, 0.300, 0.400, 0.800, 1.200, 5.000; and (b) as before but with an additional 0.500 s delay time. Spectra were recorded in a $(M_0 - M_z)$ difference mode to obtain greater accuracy on measured peak intensities before and near the null point. Furthermore, in this way, the selectivity of each experiment could be checked. Initial rates were calculated from semilogarithmic plots of $(M_0 - M_z)/2M_0$ vs. τ and by computer fitting of relaxation curves; a 3% experimental error was estimated. The probe temperature, controlled at ± 1°C by the Bruker unit, was 26 °C.

3. Results and discussion

The spin-lattice relaxation rate, R', of a proton i surrounded by other protons j at their thermal equilibrium, is in general [1] described by:

$$R' = \sum_{i \neq j, m} R'_m \tag{1}$$

where m accounts for relaxation mechanisms such as intramolecular dipole-dipole (IDD), intermolecular dipole-dipole (XDD), spin rotation (SR), chemical shift anisotropy (CSA) and scalar coupling (SC).

In a conventional nonselective spin-lattice relaxation rate measurement, the simultaneous perturbation of all the nuclei induces extensive cross-relaxation [1,9]. If only the IDD mechanism contributes to the proton relaxation process, the nonselective relaxation rate, $R'(\mathrm{NS})$, is described by [9]:

$$R'(\mathrm{NS}) = R' + \sum_{i \neq j} \sigma^{ij} \tag{2}$$

where the σ terms are the cross-relaxation rates.

Freeman et al. [9] showed that using a $(180°(\text{selective})-\tau-90°(\text{nonselective})-T)_n$ pulse sequence and τ values short enough to consider the dipolarly interacting protons j to be still at their thermal equilibrium (initial rate approximation), the experimental $R'_0(\mathrm{SE})$ is identical to the R' rate found in the previous equations. Furthermore, by simultaneous selective excitation of only two nuclei, i and j, the new experimental relaxation rate, $R'_0(\tilde{i}, \tilde{j})$, is related to the other relaxation parameters by:

$$R'_0(\tilde{i}, \tilde{j}) = R' + \sigma^{ij} \tag{3}$$

Thus, combining monoselective and biselective relaxation rate measurements, σ^{ij} can be evaluated; the latter depends upon the interproton distance, r_{ij} and the correlation time, τ_c^{ij}, which describes the reorientation of the internuclear vector, according to the equation:

$$\sigma^{ij} = \frac{\hbar^2 \gamma^4}{10} \left(r_{ij}^{-6}\right) \left(\frac{6\tau_c}{1 + 4\omega_0^2 \tau_c^{ij\,2}} - \tau_c^{ij} \right) \tag{4}$$

As shown in fig. 1, from cross-relaxation rates it is possible to obtain structural and/or motional information.

3.1. The F' ratios

These ratios, defined as:

$$F' = R^i(\mathrm{NS})/R'_0(\mathrm{SE}) = 1 + \left[\frac{6\tau_c}{1 + 4\omega_0^2 \tau_c^2} - \tau_c \right] \cdot \left[\tau_c + \frac{3\tau_c}{1 + \omega_0^2 \tau_c^2} + \frac{6\tau_c}{1 + 4\omega_0^2 \tau_c^2} \right] \tag{5}$$

range from 1.5 to 0 depending on the molecular motion and the effective spin-lattice relaxation mechanism [10].

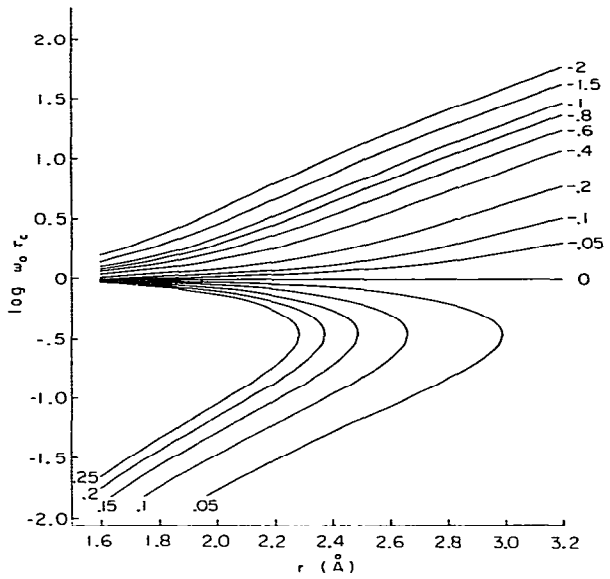

Fig. 1. Isosigma contours for $\log \omega_0 \tau_c$ vs. interproton distances calculated from eq. 4; the selected σ values are expressed in s^{-1}.

Except for ornithine (Orn) Hα, the F^i ratios for all the α-protons are 0.74 ± 0.04, as reported in table 1; using this value and eq. 5, an average correlation time of $1.2 \pm 0.1 \times 10^{-9}$ s is obtained,

Table 1

Proton spin-lattice relaxation rates and F^i ratios of gramicidin S

		δ [a]	R^i(NS) [b]	R^i_0(SE) [b]	F^i
Pro	H_{δ_1}	3.80	3.44	4.91	0.70
	H_{δ_2}	2.80	3.34	4.84	0.70
Orn	H_α	4.92	2.56	3.14	0.82
	NH	8.22	2.78	3.63	0.76
Phe	H_α	4.50	1.82	2.43	0.74
	NH	8.90	2.73	3.58	0.76
Leu	H_α	4.73	1.93	2.48	0.77
	NH	8.55	2.86	3.69	0.77

[a] Chemical shift in ppm from internal TMS.

[b] Initial relaxation rates (in s^{-1}), calculated from semilog arithmic plots of $(M_z - M_0)/2M_0$ vs. delay times, τ, of the $(180° - \tau - 90° - T)_n$ pulse sequence. Experimental errors in relaxation rates are less than 5%.

in satisfactory agreement with values derived from ^{13}C-NT$_1$ values at 15 MHz [19] and 65 MHz [20], and from values calculated from combined {^{1}H : ^{1}H} NOEs and proton relaxation rates [11]. The C_2 symmetry of gramicidin S, as reflected in its NMR spectrum [12], means that any proton resonance arises from two chemically and magnetically equivalent nuclei located on residues i and $i + 5$, respectively. In this case, any monoselective excitation experiment gives spin-lattice relaxation rates that still include cross-relaxation contributions if the equivalent protons are dipolarly coupled. This is possible only for the α-protons of Orn^2 and Orn^7 residues, since all the other backbone protons are too distant from their equivalent to be efficiency dipolarly coupled. The high F^i ratio found for Orn Hα reflects the transannular interaction. By assuming F^i should be 0.74 for these protons, the true R^i (SE) was obtained from R^i (NS)/0.74. Thus, the corrected value for Orn Hα is R^i (SE) $= 3.4 \pm 0.1$ s^{-1}.

3.2. Cross-relaxation rates, r_Φ and r_Ψ interproton distances, chain folding distances and correlation times

Two cross-relaxation rates, σ^{ij} and σ^{ji}, were obtained for several ij vectors of gramicidin S using the equations:

$$\sigma^{ij} = R^i(\tilde{i}, \tilde{j}) - R^i(\mathrm{SE}) \quad (6)$$

$$\sigma^{ji} = R^j(\tilde{i}, \tilde{j}) - R^j(\mathrm{SE}) \quad (7)$$

These parameters yield information [9,10] on (i) correlation times from calibration r_{ij} vectors, (ii) r_Φ, r_Ψ and any other unknown interproton distance when τ_c is known. The data shown in table 2 indicate a general good similarity of σ^{ij} and σ^{ji}, supporting the reliability of the calculated cross-relaxation rates and the fact that a good selectivity of the 180° pulse was achieved. A further indication of the accuracy and reasonableness of the reported σ comes from the fact that they agree with similar values obtained previously with different methods [2,3]. The slightly higher σ values of the present report arise from the higher concentration of gramicidin S used here. It is well known that the viscosity of a solution is highly affected by

Table 2

Cross-relaxation rates, distances and correlation times for interproton vectors of gramicidin S in DMSO-d_6

Hi	Hj	(σ^{ij}) [a]	(σ^{ji}) [b]	$(\langle\sigma^{ij}\rangle)$ [c]	(r^{ij}) [d]	(d^{ij}) [e]	(τ_c^{ij}) [f]	(σ^{ij}) [g]
Pro H_{δ_1}	Pro H_{δ_2}	−1.35	−1.50	−1.4	–	1.77	1.2×10^{-9}	−0.77
Orn NH	Val H_α	−0.49	–	–	2.1	–	–	−0.036
Leu NH	Leu H_α	−0.06	−0.11	−0.08	2.9	2.95	1.3×10^{-9}	−0.06
Leu NH	Orn H_α	−0.33	−0.36	−0.34	2.3	–	–	−0.32
Phe NH	Leu H_α	−0.36	−0.36	−0.36	2.2	–	–	−0.30
Phe NH	Phe H_α	−0.08	–	–	2.8	2.84	1.2×10^{-9}	−0.06
Orn NH	Orn H_α	−0.07	−0.09	−0.08	2.8	2.96	1.2×10^{-9}	−0.04

[a] Cross-relaxation rate (s^{-1}) from $R^i(i,j)-R^i$(SE).
[b] Cross-relaxation rate (s^{-1}) from $R^j(i,j)-R^j$(SE).
[c] Averaged σ values from the two differences.
[d] Interproton distances calculated by using $\tau_c = 1.2\times10^{-9}$ s and averaged σ values.
[e] Interproton distances calculated by ^3J or standard bond lengths and angles.
[f] Correlation times obtained from cross-relaxation rates and d^{ij}.
[g] Cross-relaxation rates (s^{-1}) from ref. 2.

peptide concentration and, therefore, the latter largely influences τ_c. An estimate of how sensitive this can be is shown in fig. 1. Since gramicidin S and related molecules fall in the $\omega_0\tau_c > 1$ region, a 10% increase in τ_c can yield a 50% larger σ contribution, especially when the latter involves small interproton distances. The agreement in table 2 for all σ values is, therefore, satisfactory when it is considered that the investigated distances range from 1.8 to 2.9 Å.

Two classes of interproton distances were used to evaluate correlation times. A correlation time $\tau_{gem} = 1.2\times10^{-9}$ s was obtained for the Pro CH_2, δ-methylene vector. From each r_Φ interproton distance of the NH-Hα vectors for each residue [11], two correlation times were found. To distinguish these, the value of τ_c derived from F' values was used. Both F' and σ-derived τ_c values agreed with those from ^{13}C-NT_1 [19,20].

Two conclusions can be drawn that only one correlation time, $\tau_c = 1.2\pm0.1\times10^{-9}$ s, modulates the dipolar interactions among backbone protons of gramicidin S and δ-protons of the proline residue. A similar conclusion for ^{13}C-H vectors has been proposed [19,20], and the agreement between crystal distances [22] and solution distances derived from NOEs [2] confirms this.

By using the previously proposed τ_c, r_Φ and r_Ψ were calculated, as reported in table 2. These values agree with those obtained from scalar coupling constants and the Karplus curve [2]. It is worth noting that both sets of r_Φ distances were derived from complementary interactions, i.e., through-bond for scalar ^3J and through-space for relaxation parameters. As seen in table 2 and commented upon previously [18], ^3J values are generally consistent with four Φ angles and hence four r_Φ distances. These relaxation measurements therefore present a method of reducing the $^3J/\Phi$ degeneracy [18]. Previously, this was done by Ramachandran plots, heteronuclear coupling constants, hydrogen-bonding patterns or energy minimization [21]. The calculated r_Φ values agree with those previously reported [2].

3.3. Transannular interproton distances

As discussed in section 3.1, monoselective relaxation rates of ornithine α-protons are affected by cross-relaxation and a true R^i(SE) can be calculated. By comparing observed and calculated monoselective relaxation rates, the transannular cross-relaxation contribution has been estimated to be -0.3 ± 0.1 s^{-1}. From this $\sigma_{transan}$ and the molecular correlation time, an internuclear distance of 2.4 ± 0.1 Å was calculated for Orn^2 and Orn^7 α-protons. This is in good agreement with the one expected for the antiparallel β-pleated sheet.

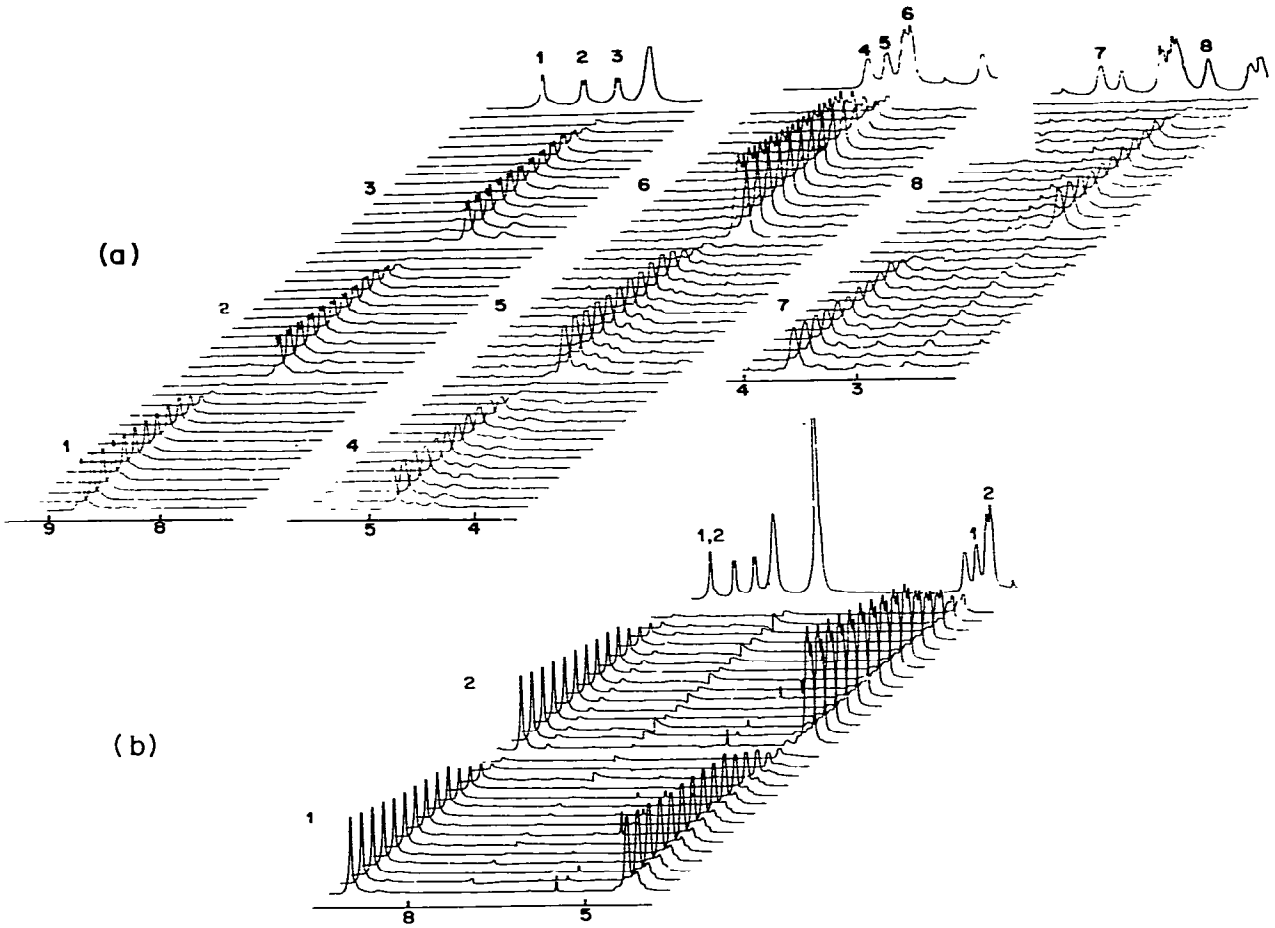

Fig. 2. (a) Monoselective and (b) biselective excitation partially relaxed spectra recorded in the ($M_0 - M_z$) mode of gramicidin S (10 mM) in DMSO-d_6 at 26 °C. In panel a, selective inversion recovery experiments are shown for Phe NH (1), Orn NH (2), Leu NH (3), Orn H (4), Leu H (5), Phe H $_\alpha$ (6), Pro H_{δ_1} (7) and Pro H_{δ_2} (8); in panel b, biselective experiments are shown for Phe NH-Leu H_α (1) and Phe NH-Phe H_α (2).

3.4. Relaxation behavior of amide protons

Amide protons of gramicidin S exhibit, in general, similar relaxation rates (see table 1) and faster than those observed for the Hα protons. This feature can be explained if the dipolar interaction with the covalently bound ^{14}N nucleus is taken into account. This additional relaxation contribution can be estimated as 0.8 s^{-1}, a value consistent with a previous analysis [22] and the correlation time here proposed.

4. Conclusion

Interproton distances and correlation times for gramicidin S have been calculated from proton spin-lattice relaxation rates assuming a pure di-

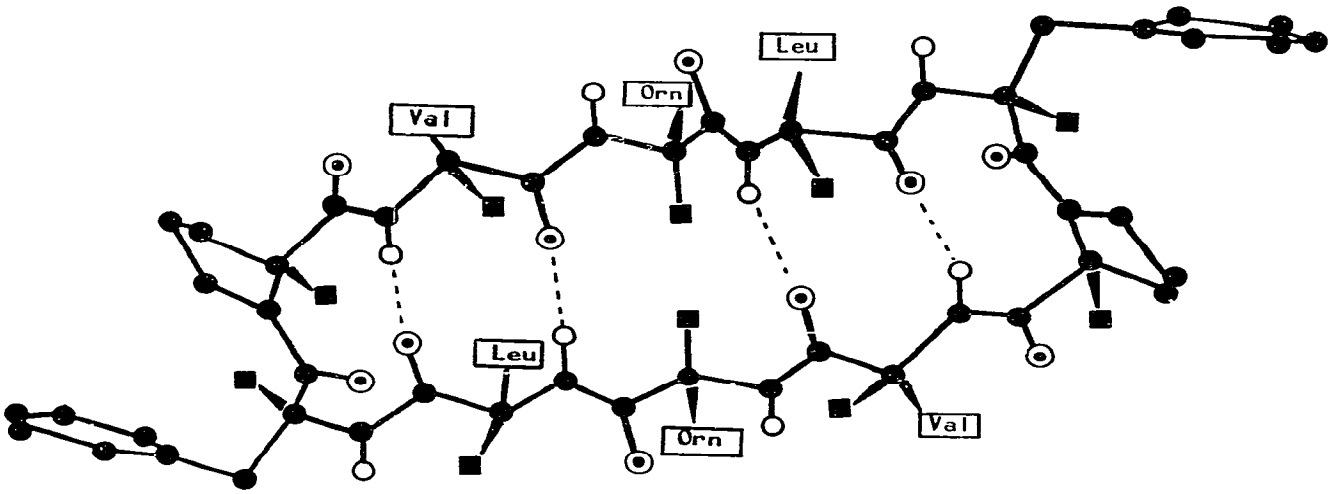

Fig. 3. Backbone conformation of gramicidin S. (○, ■, ● and ⊙) Amide hydrogen, H_α hydrogen, carbon and oxygen atoms, respectively.

polar relaxation mechanism. The calculated distances agree with those derived by crystallography [23], by combining NOEs and selective relaxation rates and by scalar coupling constants. Generally, a single (r_Φ, r_Ψ) determination is consistent with four secondary conformations per residue and hence 4^{10} are possible for a decapeptide such as gramicidin S. By measuring the transannular distance betwen the α-protons of ornithine residues, many of these 4^{10} are rejected. Furthermore, this and the other calculated distances satisfy the antiparallel β-pleated sheet/β11'-turn conformation shown in fig. 3.

Correlation times evaluated from cross-relaxation parameters and F' ratios were in agreement with each other and values previously reported [9,20,21].

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